The differences in metabolic activity of subitaneous and diapause eggs in *Centropages tenuiremis*

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The respiration and the isoenzymes of subitaneous and diapause eggs of Centropages tenuiremis in Xiamen waters of China were investigated. The results demonstrated that the average oxygen consumption of subitaneous eggs was lower than that of diapause eggs during the pre-diapause stage. The character of respiration in diapause eggs was as follows: the oxygen consumption quickly rose after spawning, and reached a maximal value, then gradually declined to a lower value that was maintained at a lower value (~20% of the maximal value), the low value of oxygen consumption in diapause eggs was lower than that in subitaneous eggs at the same temperature (~70%). The experimental results also indicated that among subitaneous, diapause and pre-diapause eggs of Centropages tenuiremis, the pre-diapause eggs were the most sensitive to temperature, the diapause eggs were the most insensitive; the sensitivity of subitaneous eggs to temperature was intermediate. The results also showed that there are obvious differences of isozyme patterns in malate dehydrogenase (MDH), sorbitol dehydrogenase (SDH), superoxide dismutase (SOD) and esterase (EST) between subitaneous eggs and pre-diapause eggs, and the characteristic enzyme bands for pre-diapause eggs were shown to be as follows: MDH^{61}, MDH^{81}, SDH^{41}, SDH^{60}, SOD^{97}, EST^{48}, EST^{59} and EST^{61}; for subitaneous eggs: MDH^{59}, SDH^{70} and EST^{93}.

INTRODUCTION

Many copepod species can produce diapause eggs to overcome periods of adverse environmental conditions. When the favorable seasons return, the diapause eggs resume their development and hatch to re-establish the population. The most remarkable character of diapause is the arrest of development and growth, and animals in diapause maintain a very low metabolic level. There have been many studies since the phenomenon of copepod diapause was first described. To date, most of these studies have been focused on the observation of morphology and timing of occurrence of diapause eggs (Li *et al.*, 1989; Ianora and Santella,1991; reviewed by Belmonte *et al.*, 1997; Dharani and Altaff, 2004), the survivability of diapause egg to critical conditions (Kasahara and Uye, 1979; Marcus, 1984; Lutz *et al.*, 1992; Parker *et al.*, 1996; Marcus and Lutz, 1998), elucidation the environmental cues inducing, controlling and terminating diapause (Marcus, 1987; Ban and Minoda, 1994; Libman and Threlkeld, 1999; Newton and Mitchell, 1999; David and Edward, 2000) and their distribution within sediments of diapause eggs (Marcus *et al.*, 1994; Marcus, 1995; Marcus and Boero, 1998; Jiang *et al.*, 2004a, b; Siokou-Frangou *et al.*, 2005; Wang *et al.*, 2005a). However, the metabolic and biochemical changes that occur during diapause in copepod embryos are largely unknown: only the biochemical character of diapause eggs in *Centropages tenuiremis* was analyzed in our laboratory (Wang *et al.*, 2005b), besides the reports of respiratory physiology of diapause in *Anomalocera patersoni* (Romano *et al.*, 1996a) and *Pontella mediterranea* (Romano *et al.*, 1996b). The studies in this

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field are necessary to understand the physiological mechanism of diapause.

The planktonic calanoid copepod *C. tenuiremis* Thompsen & Seott, 1903, is widely distributed along the coast of China (Huang, 1994). Females lay subitaneous eggs with a smooth surface or with short spines, and which hatch within 2 days, during the period December to April, and mostly diapause eggs with long spines during May and June, when the population density is lower (Li *et al.*, 1989). The species shows drastic seasonal variations in abundance and it is a dominant copepod species in Xiamen waters during winter-spring, from December to May (Chen *et al.*, 1998; Wu *et al.*, 2007). In this study, the differences in respiration, isoenzymes and comparative activities of each enzyme band between subitaneous and diapause eggs of this species were preliminarily investigated. The results will contribute to understand the differences in physiology and metabolic characteristics between the subitaneous and diapause eggs.

METHOD

Adult *C. tenuiremis* were collected from January to June 2003 using a plankton net with 300 μm mesh in the surface waters off Xiamen, China. After sampling, the contents of the tow were diluted into 15 L polythene barrels with ambient seawater and transported to the laboratory within 30 min. In the laboratory within ~30 min, the vigorous and mature females with spermatophores attached were selected using a wide-mouth pipette and incubated in incubators with 20 μm filtered seawater at the *in situ* temperature. Approximately 100 females were placed in each of the big incubators (5 L). The incubators were modified from Burkart and Kleppel (1998), and the light condition was natural light in the laboratory without direct radiation. The copepods were fed with a mixture of *Isochrysis galbana*, *Phaeodactylum tricornutum* and *Platymonas* sp. The next day, eggs were collected with a 40 μm mesh sieve, and subitaneous and diapause eggs were distinguished according to Li *et al.* (1989). The numbers of both kinds of eggs were counted, respectively, using an elongated pipette under a stereo microscope.

Respiration

Subitaneous and diapause eggs were collected at the same time (within 40 min after spawning) and from the same batch of ripe females, respectively, and can therefore be considered to be of the same age at the start of experiments. Before the experiment, the filtered seawater was sterilized, and then its salinity was regulated to 29 psu, and finally, it was put in the incubators at the experimental temperatures, 26°C or 16°C (representing typical early summer or winter temperature in the Xiamen waters).

After collection, eggs were immediately placed in small glass tubes, and then rinsed with sterilized seawater a few times. Each tube containing ~2000 embryos was covered with 40 μm mesh cloth sieve to avoid rupturing the eggs during the oxygen measurement. Just prior to each experiment, each tube containing the eggs was incubated for 20 min in 5000 U mL^{-1} Penicillin G and 7.5 mg mL^{-1} Streptomycin, and then was washed three times with sterilized seawater and placed into a 30 mL-sterilized bottle. Two replicate samples of eggs and three controls (with glass tube but without eggs) were prepared for each measurement. After 16~20 h from the start of experiments, O_2 levels of the experimental bottles and the blank bottles were measured with the WTW Oxygen sensor StirrOx G or the Winkler method with modification. Just before putting the Oxygen sensor into the bottles, the glass tubes were stuck on the bottom very carefully by a clutching pawl (made of stainless steel) to prevent its swirling with the stirring rod (Fig. 1). O_2 consumption was calculated by assuming that O_2 saturation was 100% at this temperature and at a salinity of 29 psu, and the level of controls was regarded as the initial level. Respiration measurements were taken only once in the case of subitaneous eggs, while in the case of diapause the measurements were taken once a day in the first few days and then every 2–3 days for up to the 85th day.

Isoenzyme electrophoresis

After collection of the eggs, the samples (containing 2000 eggs) were rinsed with sterilized bi-distilled water a few times, and immediately frozen and stored at −80°C in Eppendorf tubes for the isoenzyme electrophoresis.

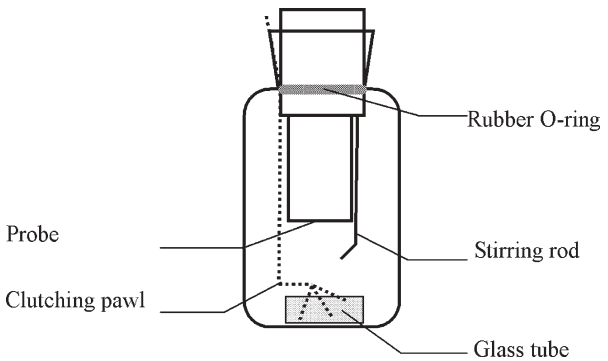

Fig. 1. Experimental setup used for measuring oxygen consumption of subitaneous and diapause eggs of *Centropages tenuiremis*.

The frozen and stored samples used for isoenzyme studies were homogenized in an ice-bath with a glass pestle in 10 μL extraction buffer [20% sucrose, 50 mM Tris–HCl (pH 7.1), 0.5% Triton X-100, 0.05% Bromphenol Blue], and then the pestle was rinsed with total 20 μL extraction buffer. The homogenate was centrifuged at 15 000 rpm for 10 min at 4°C. The supernatant (25 μL) was immediately used for electrophoresis in discontinuous vertical polyacrylamide gels with two layers, a running gel of 7.5% polyacrylamide and a stacking gel at 3% polyacrylamide. Electrophoresis was carried out in the fridge at 4°C at a constant current of 200 V, and 400 mA for a period of 5 h.

Both subitaneous and diapause embryos were initially evaluated with four isoenzyme systems: malate dehydrogenase (EC 1.1.1.37, MDH), sorbitol dehydrogenase (EC 1.1.1.14, SDH), esterase (EC 3.1.1.1, EST) and superoxide dismutase (EC 1.15.1.1 SOD), stained as detailed by Vallejos (1983), with each system repeated at least twice in order to confirm the results. The images were made using a GAS7001B Gel Image Analysis System. Variation in banding patterns was determined by the migration from the origin towards the anode. The bands were numbered from the slowest to the fastest migrating from the point of insertion of the wicks in the gel. And the Rf value and intensity for each band of the enzymes, except EST, were determined using SynGene GeneTools software (Version 3.00.22). For EST, the contrast between band (black-brown) and background (lilac) was so weak in gray-scale mode that the Rf value and intensity could not be determined using the SynGene GeneTools software. Thus, the Rf values were manually computed according to the image in our results.

RESULTS

The difference in respiration between subitaneous and diapause eggs

The oxygen consumption did not differ ($F = 1.239$, $P = 0.316$). The average oxygen consumption, expressed as ng O_2 h^{-1} $embryo^{-1}$, was a little lower (no significant difference, $t = 2.84$, $P = 0.052$) in *C. tenuiremis* subitaneous embryos (before hatching) than that in diapause embryos during the first day after spawning at 26°C (Table I). The average O_2 consumption of the subitaneous embryos incubated at 16°C was much lower than those at 26°C ($t = 4.37$, $P = 0.024$) with the value of Q_{10} (temperature coefficient) being 2.05.

Oxygen consumption for newly spawned (in the first day) diapause embryos maintained continuously at 16°C was low, only 0.56 ng O_2 h^{-1} $embryo^{-1}$, which was similar to that in subitaneous embryos. Then, it showed a rapid rise within the first few days and reached maximum uptake rates of 1.865 ng O_2 h^{-1} $embryo^{-1}$ on the fifth day (Fig. 2A), which was almost 3.5 times the average for subitaneous embryos. Thereafter, the average oxygen consumption gradually decreased, and the values were below 0.5 ng O_2 h^{-1} $embryo^{-1}$ from the 20th day to the 85th day after spawning, with the average value of

Table I: Oxygen consumption of subitaneous and diapause eggs in the first day (from 2 to 20 h after spawning)

Temperature (°C)	Subitaneous egg		Diapause egg
	Winkler method	Oxygen electrode method	Oxygen electrode method
26	0.93 ± 0.17	1.24 ± 0.23	3.39 ± 1.05
16	0.55 ± 0.04	0.51 ± 0.05	0.56 ± 0.02

Data: mean ± SD; Unit: ng O_2 h^{-1} $embryo^{-1}$.

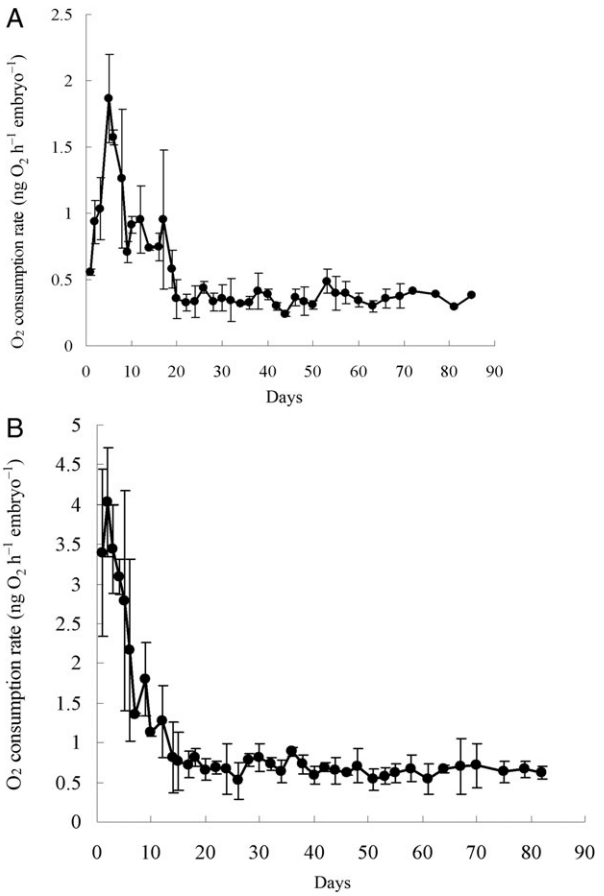

Fig. 2. *Centropages tenuiremis* diapause embryos. Rate of oxygen consumption at (**A**) 16°C and (**B**) 26°C (data points represent means ± SD).

0.38 ng O_2 h^{-1} $embryo^{-1}$, which was 72% of the average for subitaneous embryos. The lowest value was on the 44th day after spawning, 0.24 ng O_2 h^{-1} $embryo^{-1}$, only 13% of the maximum value.

Oxygen consumption for diapause embryos maintained continuously at 26°C showed a similar trend as that described for 16°C, but with higher value and more a rapid rise and decline (Fig. 2B). Respiration of diapause embryos during the first day was high, 3.39 ng O_2 h^{-1} $embryo^{-1}$, and reached a maximum on the second day, 4.04 ng O_2 h^{-1} $embryo^{-1}$, which was 3.7 times the average for subitaneous embryos. Oxygen consumption then slowly decreased, with the average value of 0.68 ng O_2 h^{-1} $embryo^{-1}$, which was 66% of the average for subitaneous embryos. All values from the 12th day to the 82nd day were below 1.00 ng O_2 h^{-1} $embryo^{-1}$. The lowest value was on the 51st day after spawning, 0.54 ng O_2 h^{-1} $embryo^{-1}$, only 13% of the maximum value (the same trend as the percentage for 16°C).

The changes in respiration of diapause eggs displayed in Fig. 2 can be summarized as follows: diapause egg respiration can be divided into two stages. In stage I, which lasted for ~12–20 days from spawning, O_2 consumption increased rapidly to reach maximum levels in the first few days and afterwards gradually decreased; in stage II oxygen consumption remained at lower value (which we called the residual metabolic level) for a long time. In addition, the average oxygen consumption of subitaneous eggs was higher than that of diapause eggs in the residual metabolic level at the same temperature conditions. The relationships between oxygen consumption and temperature demonstrate that diapause eggs have a higher Q_{10} value for the maximum level compared with the residual metabolic levels, with a Q_{10} of 2.17 compared with only 1.79.

Isoenzymatic analyses of subitaneous and diapause eggs

The isoenzyme polymorphism of the two types of eggs was analyzed with four isoenzyme systems.

MDH exhibited six bands for diapause eggs and five bands for subitaneous eggs. Band 3 (MDH^{59}) was absent in diapause eggs, whereas bands 4 (MDH^{61}) and 5 (MDH^{81}) were absent in subitaneous eggs (Fig. 3A). The bands with maximum intensity were band 4 (MDH^{61}) for diapause eggs and band 3 (MDH^{59}) for subitaneous eggs, respectively. In addition, the total intensity of MDH was ~65% higher in subitaneous eggs than in diapause eggs (Table II).

SDH exhibited seven bands for diapause eggs and six bands for subitaneous eggs. Band 8 (SDH^{70}) was absent in subitaneous eggs, whereas bands 4 (SDH^{41}) and 6 (SDH^{60}) were absent in diapause eggs (Fig. 3B). The bands with maximum intensity were band 6 (SDH^{60}) for diapause eggs and band 5 (SDH^{54}) for subitaneous eggs, respectively. In addition, the total intensity of SDH was ~15% higher in diapause eggs than in subitaneous eggs (Table II).

SOD exhibited three bands for diapause eggs and two bands for subitaneous eggs. Band 3 (SOD^{97}) was absent in subitaneous eggs (Fig. 3C). The bands with maximum intensity were band 2 (SOD^{92}) both for diapause and subitaneous eggs. The total intensity of SOD was much more similar in diapause and subitaneous eggs (Table II).

EST exhibited five bands for diapause eggs and three bands for subitaneous eggs. Bands 1 (EST^{48}), 2 (EST^{59}) and 3 (EST^{61}) were absent in subitaneous eggs, whereas band 6 (EST^{93}) was absent in diapause eggs (Fig. 3D).

DISCUSSION

A result of the present study is that it is possible to demonstrate that the sequence of metabolic changes during diapause in *C. tenuiremis* is similar to that described in *P. mediterranea* (Romano *et al.*, 1996b) and some insects (Phillips and Newsom, 1966; Okada, 1971; Braune, 1976). However, further studies should be made on copepod diapause in order to understand if the diapause of insects and copepods are governed by similar physiological processes.

Variation of respiration in diapause eggs

Respiration of an organism is an important attribute of its physiological activity and reflects the metabolism *in vivo*. It is known that during insect diapause oxygen consumption is low. While the information on the physiological differences between the diapause and subitaneous embryos of copepods is lacking, even though it has generally been assumed to be comparable to that in insects (Romano *et al.*, 1996a).

The respiration curve of diapause eggs in *C. tenuiremis* after spawning followed the typical L-shaped pattern, just like the first half of U-shaped pattern described by Romano *et al.* (1996b). There was an initial pre-diapause period representing the first part of the L-shaped curve, characterized by high rates of O_2 uptake. This was followed by a slow decrease in O_2 uptake possibly due to a progressive decrease in permeability of the fertilization envelope, as suggested by Okada (1971) and Romano *et al.* (1996b). After 20 days, in the real diapause stage, a low diapause level of respiration was reached (~20% of the maximum level of pre-diapause). It is regretted that

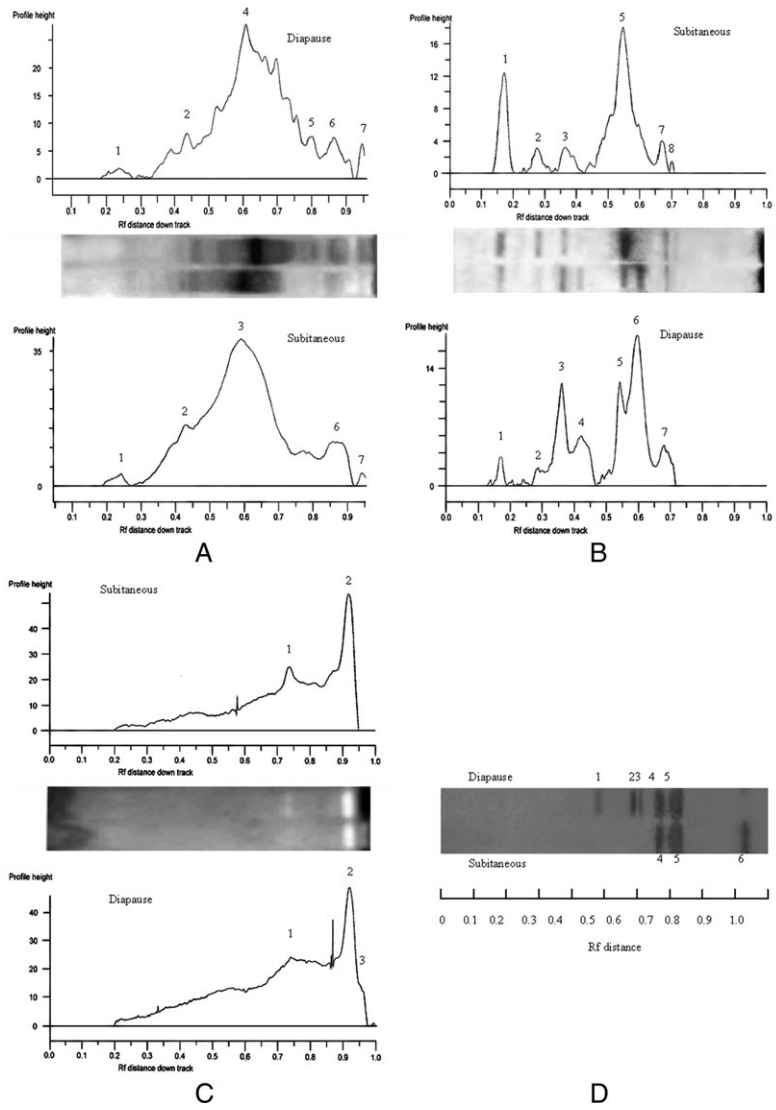

Fig. 3. Electrophoresis diagram of isozymes in subitaneous and diapause eggs of *Centropages tenuiremis* (**A**) MDH, (**B**) SDH, (**C**) SOD and (**D**) EST.

our experiments only lasted 85 day, so the respiration of embryos during post-diapause stage up to the emergence of nauplius could not be established as in other organisms, e.g. *P. mediterranea* (Romano *et al.*, 1996b). The respiratory physiology of summer diapausing eggs of the copepod *A. patersoni* was quite different from that of *C. tenuiremis* and *P. mediterranea*, and it was characterized by a bell-shaped curve with low O_2 consumption at the beginning of diapause. In the former case, the maximum respiration level was reached very late, at 70 days after spawning and was followed by a slow diminution in O_2 uptake (Romano *et al.*, 1996a). It was very clear that there were two types of diapause characteristics among the eggs of the three species mentioned above (Romano *et al.*, 1996a). The difference might result from their own ecological acclimatization to their habitat.

Studies of many organisms have shown that by reducing metabolic activity and switching to anaerobic metabolism individuals can extend survival because of the reduced demand on metabolic substrates (Guppy *et al.*, 1994). There was a correlation between the degree of metabolic depression and the duration of resting (Hand, 1998). For example, for diapause embryos of *Artemia franciscana*, the energy flow was depressed to a rather low level (2.4%) (Hand and Gnaiger, 1988). For *C. tenuiremis*, both at 16°C and 26°C, the respiration levels of diapause eggs (residual metabolic level) were ~30% lower than

Table II: Information for four isoenzymic zymograms in diapause and subitaneous eggs, Centropages tenuiremis

		Rf		Height		Relative intensity		Width	
Enzyme	Number	D	S	D	S	D	S	D	S
MDH	1	0.24	0.24	1.848	3.033	604.02	1098.21	8	8
	2	0.44	0.44	8.217	15.728	7013.49	14 639.6	21	17
	3	—	0.59	—	38.07	—	108748	—	53
	4	0.61	—	27.907	—	61 347.4	—	53	—
	5	0.81	—	7.083	—	4913.18	—	16	—
	6	0.87	0.87	7.297	11.429	4603.61	8091.68	14	13
	7	0.94	0.94	5.965	3.312	2877.02	1685.24	13	11
	Total intensity					81 358.7	134 263		
SDH	1	0.17	0.17	3.531	12.405	540.42	3437.68	9	22
	2	0.28	0.28	1.925	3.212	297.76	846.56	7	15
	3	0.36	0.36	12.222	3.282	3785.37	662.67	20	9
	4	0.41	—	5.657	—	1391.64	—	10	—
	5	0.54	0.54	12.348	18.142	3015.96	10 250	15	43
	6	0.6	—	17.855	—	8162.7	—	29	—
	7	0.67	0.67	4.889	4.103	1482.19	924.79	15	14
	8	—	0.7	—	1.563	—	80.17	—	3
	Total intensity					18 676	16 201.9		
SOD	1	0.74	0.74	24.662	25.471	12 987.5	15 655.9	11	13
	2	0.92	0.92	49.483	54.345	54 582	55 020.9	28	29
	3	0.97	—	9.801	—	3779.48	—	9	—
	Total intensity					71 349	70 676.8		
EST	1	0.48	—						
	2	0.59	—						
	3	0.61	—						
	4	0.66	0.66						
	5	0.71	0.71						
	6	—	0.93						

D, diapause eggs; S, subitaneous eggs.

the average value in subitaneous eggs, similar to the case of *P. mediterranea* (Romano *et al.*, 1996b).

For the diapause eggs of both *C. tenuiremis* and *P. mediterranea*, there was an initial pre-diapause period characterized by high O_2 uptake that was higher than the average level of subitaneous eggs. Then, this was followed by a drastic reduction to ~25% of the level during pre-diapause. This low level, called the residual metabolic level, was maintained for a long period and suggested that the eggs do not immediately enter into the real diapause stage after spawning, and some physiological process might be necessary to start the diapause. Hand and Podrabsky (2000) found that for both vertebrates and invertebrates, the metabolic arrest accompanying diapause required several days before maximal depression was achieved. They presumed that diapause required the specific expression of certain gene products, or conversely, the elimination of products via macromolecular turnover, both of which can require considerable periods of time. The maximum respiration level of pre-diapause was higher than the level of subitaneous eggs, implying higher energy requirements prior to diapause.

Effects of temperature on respiration of eggs in three different metabolic stages

The types of respiration curves of diapause embryos at two temperatures were very similar (Fig. 1), even though the one at the lower temperature (16°C) was much shallower with lower oxygen consumption. The results showed that the temperature had no qualitative effect on the eggs which went into diapause, but it could influence the metabolic intensity, i.e. the high temperature could increase the metabolic rate and then accelerate the metabolic process of preparation for diapause and reduce the time of the pre-diapause period. The Q_{10} effect on metabolic rate occurs virtually universally in animals, having a sound thermodynamic basis involving molecular motion and chemical reaction theory. The relatively low temperature coefficients (Q_{10} of 1.79 to 2.17) within the normal ectothermic values (typically 2–3) (reviewed by Nielsen *et al.*, 2007) suggest that both subitaneous and diapause eggs are comparatively insensitive to temperature change over this temperature range (16–26°C). In addition, the three different values of Q_{10} showed that the diapause eggs in the pre-diapause period are the most sensitive to temperature

change, then subitaneous eggs, and the diapause eggs truly in diapause stage are the most insensitive.

Enzymatic differences between subitaneous and diapause eggs

Isoenzymes, as one of the products of gene expression, have often been used as markers in genetics to study development and differentiation in breeding. Changes of isoenzyme patterns could reflect gene expression or even gene changes, and thus have contributed to the study of animal biology by providing methods for detecting differences among individuals. Isoenzymes and other biochemical characteristics are closer to the genetic information than the morphological traits. The investigation of isoenzymes would be helpful to reveal the special metabolic processes of the given developmental stages. Therefore, they have been useful for diapause characterization as shown in several insect species where a high number of bands, evaluated as qualitative characters (presence and absence), including *Teleogryllus commodus* (Jamieson *et al.*,1976), *Tetranychus viennensis* (Gotoh *et al.*, 1991) and *Bombyx mori* (Yaginuma and Yamashita, 1979) among others.

MDH is an important enzyme in the tricarboxylic acid cycle (TCA cycle) that catalyzes the conversion of malate into oxaloacetate (using NAD^+) and vice versa. The zymogram for MDH revealed the difference between the two types of eggs both in gene expression and enzyme activity. The common bands, MDH^{24}, MDH^{44}, MDH^{87} and MDH^{94}, might come from the enzyme that participated in basal metabolism. The character band in subitaneous eggs, MDH^{59}, could correspond to the metabolism for embryonic development, whereas the character band in diapause eggs, MDH^{61}, could be interpreted as a specific metabolism to prepare for entry into the real diapause stage. The higher total relative intensity of MDH in subitaneous eggs suggested that the metabolic level of the TCA cycle was higher in a single subitaneous egg than in a diapause egg. This was very similar to *B. mori* diapause egg (Kageyama and Ohnishi, 1971). In that case, most of the enzyme activities concerning the TCA cycle are low during diapause. The oxygen consumption of a single subitaneous egg was lower than that of a pre-diapause egg in our respiration experiments. So we presumed that the glycol-metabolism might not be the only main pathway (TCA cycle) in pre-diapause egg, i.e. there might be another important pathway, e.g. pentose phosphopentose pathway (also named hexose monophosphate shunt). This pathway has been found in some insect diapause egg. Kageyama (1976) calculated that the relative metabolic rate in pentose phosphopentose pathway could reach to 38% in the diapause egg of *B. mori*.

SDH is indispensable in the initial steps in the polyol pathway, a bypass to glycolysis, together with aldose reductase (Niimi *et al.*, 1993). It was paid much attention by many scientists researching diapause eggs of *B. mori*, because SDH was shown to relate to the induction and termination of diapause in *B. mori* (Yaginuma and Yamashita, 1979; Niimi *et al.*, 1992, 1993; Horie *et al.*, 2000). The zymogram for SDH revealed the difference between the two types of eggs both in gene expression and enzyme activity. The higher total relative intensity of SDH in diapause eggs suggested that SDH might participate in the induction of diapause. So the common bands, SDH^{17}, SDH^{28}, SDH^{36}, SDH^{54} and SDH^{67}, might come from the enzyme that participated in basal metabolism. The characteristic bands in diapause eggs, SDH^{41} and SDH^{60}, could be interpreted as a specific metabolism related to induction into diapause.

ESTs are enzymes capable of hydrolyzing esters that are present in all kinds of organisms. Most ESTs may have broad biological functions, and they have been extensively studied in insects and are involved in different physiological processes, such as diapause (Isobe *et al.*, 2006). The observed EST substrate-specific polymorphisms reflect the genomic expression diversity present in different developmental and physiological processes of *C. tenuiremis* embryos. Jamieson *et al.* (1976) found that ESTs related to embryonic diapause in *T. commodus*. Differences in EST zymograms were also found between non-diapausing and diapausing individuals of *T. viennensis* (Gotoh *et al.*, 1991). According to our results, the zymograms between subitaneous and diapause embryos were quite different. The common bands, EST^{66} and EST^{71}, might mainly participate in the metabolic activity in cells. No catabolism was observed in yolk platelets of diapause eggs during the first 2 weeks after spawning (Romano *et al.*, 1996b). The characteristic band of subitaneous eggs in our results, EST^{93}, might come from the enzymes that participated in the catabolic process of yolk. Furthermore, Lu and Jiang (2000) also suggested that the band of EST closest to the anode might correspond to yolk catabolism in embryo development of *Macrobrachium rosenbergh*. The characteristic bands in diapause eggs, EST^{48}, EST^{59} and EST^{61}, could be interpreted as a specific metabolism of induction into diapause, but the real metabolic function and mechanism for the above three enzymes is still unknown and require further investigation.

The collection of diapause eggs for isoenzymes analysis was completed within 2 days from deposited in our experiments, so these diapause eggs were in a pre-diapause stage as concluded from respiratory experiments. Thus, the analysis of isoenzymes reflected the physiological difference between eggs in subitaneous

and pre-diapause stages, not in subitaneous and real diapause stages.

The four enzymatic activities were high in the diapause embryos, and there was no metabolic rate depression during the first 2 days from deposition. This was consistent with the result from respiration experiment above. From the isoenzymes pattern, we presumed that the metabolic processes might be more complicated in pre-diapause stage.

In addition, a limitation of our experiment was the neglect of temperature effect on the enzymatic activities, since the two types of eggs in isoenzymes analysis came from two different seasons (subitaneous eggs collected in February and diapause eggs in May).

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REFERENCES

Ban, S. and Minoda, T. (1994) Induction of diapause egg production in *Eurytemora affinis* by their own metabolites. *Hydrobiologia*, **293**, 185–189.

Belmonte, G., Miglietta, A., Rubino, F. *et al.* (1997) Morphological convergence of resting stages of planktonic organisms: a review. *Hydrobiologia*, **355**, 159–165.

Braune, H. J. (1976) Effects of temperature on the rates of oxygen consumption during morphogenesis and diapause in the egg stage of *Leptopterna dolobrata* (Heteroptera, Miridae). *Oecologia*, **25**, 77–87.

Burkart, C. A. and Kleppel, G. S. (1998) A new incubation system for the measurement of copepod egg production and egg hatching success in the field. *J. Exp. Mar. Bio. Ecol.*, **221**, 89–97.

Chen, R. X., Lin, J. H., Lin, M. *et al.* (1998) Ecological study on zooplankton in western Xiamen Harbour. *J. Oceanogr. Taiwan Strait*, **17**, 294–298 (in Chinese with English abstract).

David, W. P. and Edward, J. M. (2000) Factors influencing the induction of diapausing egg production in the calanoid copepod *Diaptomus leptopus*. *Aquat. Ecol.*, **34**, 9–17.

Dharani, G. and Altaff, K. (2004) Ultra structure of subitaneous and diapausing eggs of planktonic copepod Sinodiaptomus (Rhinediaptomus) indicus. *Curr. Sci.*, **87**, 109–112.

Gotoh, T., Ishikawa, Y., Takayama, K. *et al.* (1991) Differences in esterase zymograms between non-diapausing and diapausing individuals of the hawthorn spider mite *Tetranychus viennensis* Zacher (Acari: Tetranychidae). *Appl. Entomol. Zool.*, **26**, 153–155.

Guppy, M., Fuery, C. J. and Flanigan, J. E. (1994) Biochemical principles of metabolic depression. *Comp. Biochem. Physiol. B*, **109**, 175.

Hand, S. C. (1998) Quiescence in *Artemia franciscana* embryos: reversible arrest of metabolism and gene expression at low oxygen levels. *J. Exp. Biol.*, **201**, 1233–1242.

Hand, S. C. and Gnaiger, E. (1988) Anaerobic dormancy quantified in *Artemia* embryos: a calorimetric test of the control mechanism. *Science*, **239**, 1425–1427.

Hand, S. C. and Podrabsky, J. E. (2000) Bioenergetics of diapause and quiescence in aquatic animals. *Thermochim. Acta*, **349**, 31–42.

Horie, Y., Kanda, T. and Mochida, Y. (2000) Sorbitol as an arrester of embryonic development in diapausing eggs of the silkworm *Bombyx mori*. *J. Insect Physiol.*, **46**, 1009–1016.

Huang, Z. G. (ed.) (1994) *Marine Species and Their Distribution in China's Seas*. China Ocean Press, Beijing (in Chinese).

Ianora, A. and Santella, L. (1991) Diapause embryos in the neustonic copepod *Anomalocera patersoni*. *Mar. Biol.*, **108**, 387–394.

Isobe, M., Kai, H., Kurahashi, T. *et al.* (2006) The molecular mechanism of the termination of insect diapause, part 1: a timer protein, TIME-EA4, in the diapause eggs of the silkworm *Bombyx mori* is a metallo-glycoprotein. *Chem. Bio. Chem.*, **7**, 1590–1598.

Jamieson, G. W., MacFarlane, J. R. and Hogan, T. W. (1976) Esterases in relation to embryonic diapause in the field cricket *Teleogryllus commodus*. *Insect Biochem.*, **6**, 59–64.

Jiang, X., Wang, G., Li, S. *et al.* (2004a) Preliminary study on the potential recruitment of the copepods egg bank into the planktonic population in Xiamen harbor. *J. Xiamen Univ. Nat. Sci.*, **4**, 233–236 (in Chinese with English abstract).

Jiang, X., Wang, G. and Li, S. (2004b) Age, distribution and abundance of viable resting eggs of *Acartia pacifica* (Copepoda: Calanoida) in Xiamen Bay, China. *J. Exp. Mar. Biol. Ecol.*, **312**, 89–100.

Kageyama, T. (1976) Pathways of carbohydrate metabolism in the eggs of the silkworm, *Bombyx mori*. *Insect Biochem.*, **6**, 507–511.

Kageyama, T. and Ohnishi, E. (1971) Carbohydrate metabolism in the eggs of the silkworm I. absence of phosphofructokinase in the diapausing egg, *Bombyx mori*. *Dev. Growth Differ.*, **13**, 97–100.

Kasahara, S. and Uye, S. (1979) Calanoid copepod eggs in sea-bottom muds, V: seasonal changes in hatching of subitaneous and diapause eggs of *Tortanus forcipatus*. *Mar. Biol.*, **55**, 63–68.

Li, S. J., Chen, F. and Wang, G. Z. (1989) Studies on the feature of eggs and its hatching rates of some planktonic copepods in Xiamen waters. *J. Xiamen Univ. Nat. Sci.*, **28**, 538–543 (in Chinese with English abstract).

Libman, B. S. and Threlkeld, S. T. (1999) Diapause phenology and egg bank dynamics of a calanoid copepod in floodplain habitats. *Archiv. Hydrobiol.*, **145**, 349–358.

Lu, J. and Jiang, N. (2000) The isozyme patterns in embryo development of the freshwater prawn *Macrobrachium rosenbergh*. *Donghai Mar. Sci.*, **18**, 34–39 (in Chinese with English abstract).

Lutz, R., Marcus, N. H. and Chapman, J. P. (1992) Effects of low oxygen concentrations on the hatching and viability of eggs of marine calanoid copepods. *Mar. Biol.*, **144**, 241–247.

Marcus, N. H. (1984) Recruitment of copepod nauplii into the plankton: importance of diapause eggs and benthic processes. *Mar. Ecol. Prog Ser.*, **15**, 47–54.

Marcus, N. H. (1987) Differences in the duration of egg diapause of *Labidocera aestiva* (Copepoda: Calanoida) from the Woods Hole, Massachusetts region. *Biol. Bull.*, **173**, 169–177.

Marcus, N. H. (1995) Seasonal study of planktonic copepods and their benthic resting eggs in northern California coastal waters. *Mar. Biol.*, **123**, 459–465.

Marcus, N. H. and Boero, F. (1998) Production and plankton community dynamics in coastal aquatic systems: the importance of benthic-pelagic coupling and the forgotten role of life cycles. *Limnol. Oceanogr.*, **43**, 763–768.

Marcus, N. H. and Lutz, R. V. (1998) Longevity of subitaneous and diapause eggs of *Centropages hamatus* (Copepoda: Calanoida) from the northern gulf of Mexico. *Mar. Biol.*, **131**, 249–257.

Marcus, N. H., Lutz, R. V., Burnett, W. *et al*. (1994) Age, viability, and vertical distribution of zooplankton resting eggs from an anoxic basin: evidence of an egg bank. *Limnol. Oceanogr.*, **39**, 154–158.

Newton, G. M. and Mitchell, B. D. (1999) Egg dormancy in the Australian estuarine-endemic copepods *Gippslandia estuarina* and *Sulcanus conflictus*, with reference to dormancy of other estuarine fauna. *Mar. Freshw. Res.*, **50**, 441–449.

Nielsen, P., Larsen, L. H., Ramløv, H. *et al*. (2007) Respiration rates of subitaneous eggs from a marine calanoid copepod: monitored by nanorespirometry. *J. Comp. Physiol. B*, **177**, 287–296.

Niimi, T., Yamashita, O. and Yaginuma, T. (1992) Activity of NAD sorbitol dehydrogenase is caused by biosynthesis of enzyme protein in diapause and non-diapause eggs of the silkworm, *Bombyx mori*. *Comp. Biochem. Physiol. B*, **103**, 657–661.

Niimi, T., Yamashita, O. and Yaginuma, T. (1993) A cold-inducible *Bombyx* gene encoding a protein similar to mammalian sorbitol dehydrogenase. *Eur. J. Biochem.*, **213**, 1125–1131.

Okada, M. (1971) Role of the chorion as a barrier to oxygen in the diapause silkworm, *Bombyx mori*, L. *Experientia*, **27**, 658–660.

Parker, B. R., Wilhelm, F. M. and Schindler, D. W. (1996) Recovery of *Hesperodiaptomus* population from diapausing eggs following elimination by stocked salmonids. *Can. J. Zool.*, **74**, 1292–1297.

Phillips, J. R. and Newsom, L. D. (1966) Diapause in *Heliothis zea* and *Heliothis virescens* (Lepidoptera: Noctuidae). *Ann. Ent. Soc. Am.*, **59**, 154–159.

Romano, G., Ianora, A. and Miralto, A. (1996a) Respiratory physiology in summer diapause embryos of the neustonic copepod *Anomalocera patersoni*. *Mar. Biol.*, **127**, 229–234.

Romano, G., Ianora, A., Santella, L. *et al*. (1996b) Respiratory metabolism during embryonic subitaneous and diapause development in *Pontella mediterranea* (Crustacea Copepoda). *J. Comp. Physiol. B*, **166**, 157–163.

Siokou-Frangou, I., Zervoudaki, S., Kambouroglou, V. *et al*. (2005) Distribution of mesozooplankton resting eggs in seabottom sediments of Thermaikos Gulf (NW Aegean Sea, Greece) and possible effects of sediment resuspension. *Cont. Shelf Res.*, **25**, 2597–2608.

Vallejos, C. E. (1983) Enzyme activity staining. In Tanksley, S. D. and Orton, T. J. (eds), *Isoenzymes in Plant Genetics and Breeding Part A*. Elsevier, Amsterdam, pp. 469–516.

Wang, G., Jiang, X., Li, S. *et al*. (2005a) A potential source of recruitment of *Acartia pacifica* nauplii: viable benthic resting eggs. *Acta Oceanol. Sin.*, **24**, 151–158.

Wang, G., Jiang, X., Wu, L. *et al*. (2005b) Differences in the density, sinking rate and biochemical composition of *Centropages tenuiremis* (Copepoda: Calanoida) subitaneous and diapause eggs. *Mar. Ecol. Prog Ser.*, **288**, 165–171.

Wu, L. S., Wang, G. Z., Jiang, X. D. *et al*. (2007) Seasonal reproductive biology of *Centropages tenuiremis* (Copepoda) in Xiamen waters, Peoples Republic of China. *J. Plankton Res.*, **29**, 437–446.

Yaginuma, T. and Yamashita, O. (1979) NAD-dependent sorbitol dehydrogenase activity in relation to the termination of diapause in eggs of *Bombyx mori*. *Insect Biochem.*, **9**, 547–553.